~~194~~. The method of claim 193, wherein Th1 cells are produced.~~—~~

~~195~~. ~~The method of claim 193, wherein Th2 cells are produced.~~

Please amend claim 158 as follows:

158. (Amended) The method of claim 155, wherein the regulatory cells [express] are specific for a selected antigen.

REMARKS

Any fees, including fees for additional claims, that may be due with this paper or with this application during its entire pendency may be charged to Deposit Account No. 08-1641. If a Petition for extension of time is needed, this paper is to be considered such Petition. A change of address accompanies this response.

A change of address for the undersigned also accompanies this response.

Claims 11-17, 22-35 and 154-192 are presently pending. Claim 158 is amended herein to correct an inadvertent obvious error in the previous response. Regulatory cells are specific for an antigen. Claims 193-195 are added herein in or to particularly point out and distinctly claim subject matter that applicant regards as the invention.

REJECTION OF CLAIMS 1, 3-7, 11, 12, 14-17, 22-25, 31, 32, 34 and 35 UNDER 35 U.S.C. § 102(b)

Claims 1, 3-7, 11, 12, 14-17, 22-25, 31, 32, 34 and 35 are rejected under 35 U.S.C. § 102(b) as being anticipated by June et al. (WO 94/29436). The arguments in the previous response are herein incorporated in their entirety. Clarification of certain points is set forth below.

The instant claims and application are directed to methods of producing clinically relevant numbers of cells and methods for producing populations of cells that are sufficiently stable and homogeneous to be useful for clinical application.

Discl sur f June et al.

June et al. discloses a method for inducing a population of T cells to proliferate by providing a first signal to activate the cells and then a second signal to stimulate proliferation. This is essentially all that June et al. shows. Cell densities substantially greater than 10^6 are not disclosed, nor are populations of cells produced that could be clinically used.

June et al. does not disclose or teach a method in which selected populations of cells are produced. June et al. does not disclose expansion of cells to produce stable populations of effector cells (as defined in the instant specification i.e. TIL cells, LAK cells etc.), nor does June et al. disclose production of stable populations of regulatory immune cells. As defined and described in the instant specification (see, e.g., page 19):

> regulatory immune cells that produce IL-2 and IFN-γ, but do not produce IL-4 are Th1" cells. Regulatory immune cells that produce IL-4 and IL-10, but do not produce IFN-γ are termed "Th2" cells. Regulatory immune cells that produce TGF-β, IL-10 and IFN-γ, but do not produce IL-2 or IL-4 are termed "Th3" cells. Populations of cells that produce a majority of Th1 cytokines are designated "Th1-like"; populations producing a majority of the Th2 cytokines are designated Th2-like"; those producing a majority of Th3 cytokines are designated "Th3-like". Thus, each composition, although containing a heterogeneous population of cells, will have the properties that are substantially similar, with respect to cytokine, to the particular Th subset.

Example 6 on pages 31-32 of June et al. shows that the cytokine profiles produced by the cells are not characteristic of a population of cells that fit any of the above profiles, nor do the populations appear to be stable, since the lymphokine profile changes over time and the cells end up producing an array of cytokines characteristic of mixtures of cells. Thus, June et al. does not disclose a method in which the starting population is directed to a selected stable population that could be used for clinical treatment as described in the instant application. Therefore, for these reasons and the reasons of record, June et al. does not anticipate any of the instant claims. In addition to the above-noted

claims, added claims 193-195 clearly capture a distinction between the instant application and the disclosure of June et al.

THE REJECTION OF CLAIMS 1-17, 22-32 AND 31-35 UNDER 35 U.S.C. § 103(a)

CLAIMS 1, 3-7, 11-17, 22-25, and 31-35

Claims 1, 3-7, 11-17, 22-25 and 31-35 are rejected under 35 U.S.C. § 103 as being unpatentable over June et al., in view of Cracauer et al. (US patent No. 4,804,628). It is alleged that:

> June et al. teach the method of claim 1 wherein unfractionated T cells or CD4+ or CD8+ cells are expanded to clinically relevant numbers by treatment with antiCD3 antibody followed by antiCD28 or antiCTLA4 antibody . . . June et al. teach a method that can be used to produce "regulatory cells" such as CD4+ cells (see page 6). Said CD4+ cells have a defined cytokine production profile, wherein said cytokine production profile does not directly mediate an effector function (e.g., said cells produce IL-4 which can cause Th2 differentiation). Said CD4+ cells are treated to alter their cytokine production profile prior to production in that they are separated from CD8 cells (see claim 30).

Cracauer et al. allegedly teaches hollow fiber bioreactors and the use of such devices for efficiently growing large numbers of cells in vitro. It is concluded that one of ordinary skill in the art would have been motivated to do the aforementioned because Cracauer et al. teach that "hollow fiber culture devices have been proven to be ideal for the maintenance of many types of cells at high densities in culture."

The rejection is respectfully traversed for the reasons of record and for the following reasons.

June et al.

First as noted in the instant application, $CD4^+$ cells can be sub-divided according to their cytokine expression profiles. These cells are derived from a common precursor, Th0, which can produce Th1, Th2 and Th3 cytokines [see, Firestein, et al. (1989) J. Immunol. 143:518]. June et al. does not teach differentiating the $CD4^+$ cells into a selected subtype prior to expansion.

June et al. teaches a method for inducing a population of T cells to proliferate by providing a first signal to activate the cells and then a second signal to stimulate proliferation. The reference teaches use of antibodies to CD3 or CD2 to activate the cells and antibodies to CD28 to stimulate cell proliferation. June et al. also teaches purifying populations of CD4+ or CD8+ T cells and growing the cells.

June et al. does not teach the step of expanding the cells to therapeutically useful or clinically relevant numbers, nor does June et al. teach or suggest a method in which cells are differentiated. June et al. states that T cells can be grown in long term tissue culture to obtain a population increased in number from about 100 to about 100,000 fold over the original starting cell population, but does not suggest preparation of clinically relevant numbers or therapeutically effective concentrations of particular cell types.

June et al. also teaches the use of the T cell proliferation method for obtaining a population of CD4+ or $CD8^+$ T cells and, in the case of CD4+ cells, June et al. evaluates the cytokines produced by the cells at different stages of growth. This teaching, however, says nothing about the production of regulatory T lymphoid cells, and in fact the resulting populations of CD4+ cells are heterogeneous.

As noted previously, June et al. does not teach, suggest or contemplate differentiation of cells into regulatory cells. As discussed above, there is no reference to "regulatory" cells at page 6 of June et al. as alleged or anywhere else in the reference. Stimulation of CD4+ cells with this combination of antibodies used by June et al. does not generally produce regulatory cells and June et al. admits that the expanded CD4+ cells have an unstable cytokine profile (see page 31, lines 26-29).

Cracauer _t al.

Cracauer et al. teaches a hollow fiber cell culture device that includes a hollow fiber cartridge having a shell and a plurality of capillaries extending through the shell with at least some capillaries having semi-permeable walls. A cell culturing space is located between the shell and the capillaries. The device includes a chamber containing a second medium supply fluidly connected to the cell culturing space.

Cracauer et al. does not teach or suggest use of its hollow fiber cell culture for growing clinically relevant numbers of immune cells or T lymphoid cells, or for that matter any type of lymphoid cell. Cracauer et al. does not teach or suggest anything about expanding regulatory T lymphoid cells or how to grow such cells under conditions that produce high cell density.

In fact, the device of Cracauer et al., which is designed for producing monoclonal antibodies, is not effective for producing the high densities of cells required by the claims. The device of Cracauer et al. is not taught to be suitable for growing lymphoid cells at densities exceeding 1×10^8 cells/ml (1×10^9 cells/Liter) because it is not designed to maintain a high concentration of lymphokines around the cells while retaining sufficient quantities of oxygen because oxygen diffusion is still limited, however, and gradients still occur. As a result, the instantly claimed methods cannot employ the device of Cracauer et al.

June et al. does not contemplate preparation of high densities of cells nor high densities of selected populations of cells. Cracauer et al. provides no suggestion for growing T lymphoid cells in its device. Therefore, the ordinarily skilled artisan would not have had any motivation to have added an additional step to the method of June et al. and have expanded the cells in the device of Cracauer et al.

Th combination of r fer nces does not result in the claim d subj ct matter

June et al. teaches a method of growing T lymphocytes, CD4+ and CD8+ T cells at relatively low cell densities of about 1 x 10^5 cells/ml- 0.5 x 10^6 cells/ml using a combination of mitogenic antibodies in the absence of IL-2. June et al., however, does not teach or suggest a method for obtaining clinically relevant numbers of T lymphoid cells nor expansion of such cells under conditions that produce high cell density, an element recited in all of the claims. Furthermore, with respect to certain claims (see, e.g., claim 192) June et al. does not teach or suggest growth under conditions in which differentiated populations are produced.

With respect to claims 17, 22-25 and 31-35, all of which require expansion of regulatory T cells, June et al. also does not teach or suggest a method of producing regulatory T cells nor a method whereby the regulatory T cells are expanded under conditions that produce high cell density to result in clinically relevant numbers of cells. At most, June et al. teaches activation and growth of CD4+ cells using anti-CD3 + anti-CD28 and evaluation of the cytokines produced. These cells do not have a stable cytokine profile (see page 31, lines 26-29), and represent a heterogeneous population. As discussed above, simple measurement of cytokine production does not meet the definition of a regulatory immune cell as used in the specification (page 19, lines 4-19).

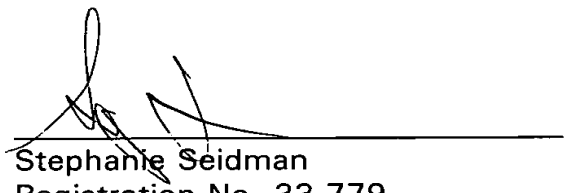
Cracauer et al. does not cure the deficiencies in the teachings of June et al. because Cracauer et al. merely teaches a hollow fiber device, that does not provide adequate oxygenation. There is no teaching or suggestion in Cracauer et al. to use the device for expanding T cells at high cell density nor how to adapt the device to achieve such a goal. Further, Cracauer et al. does not teach or suggest a method for producing regulatory cells.

Therefore, the combination of references does not teach or suggest the instantly claimed methods.

* * *

In view of the above remarks and the amendments and remarks of record, consideration and allowance of the application are respectfully requested.

Respectfully submitted,
HELLER EHRMAN WHITE & MCAULIFFE

By: 
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